CLEAN WATER (9) WATER POLLUTION CONTROL RESEARCH SERIES • 16020 FEN 03/71

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W72-03784

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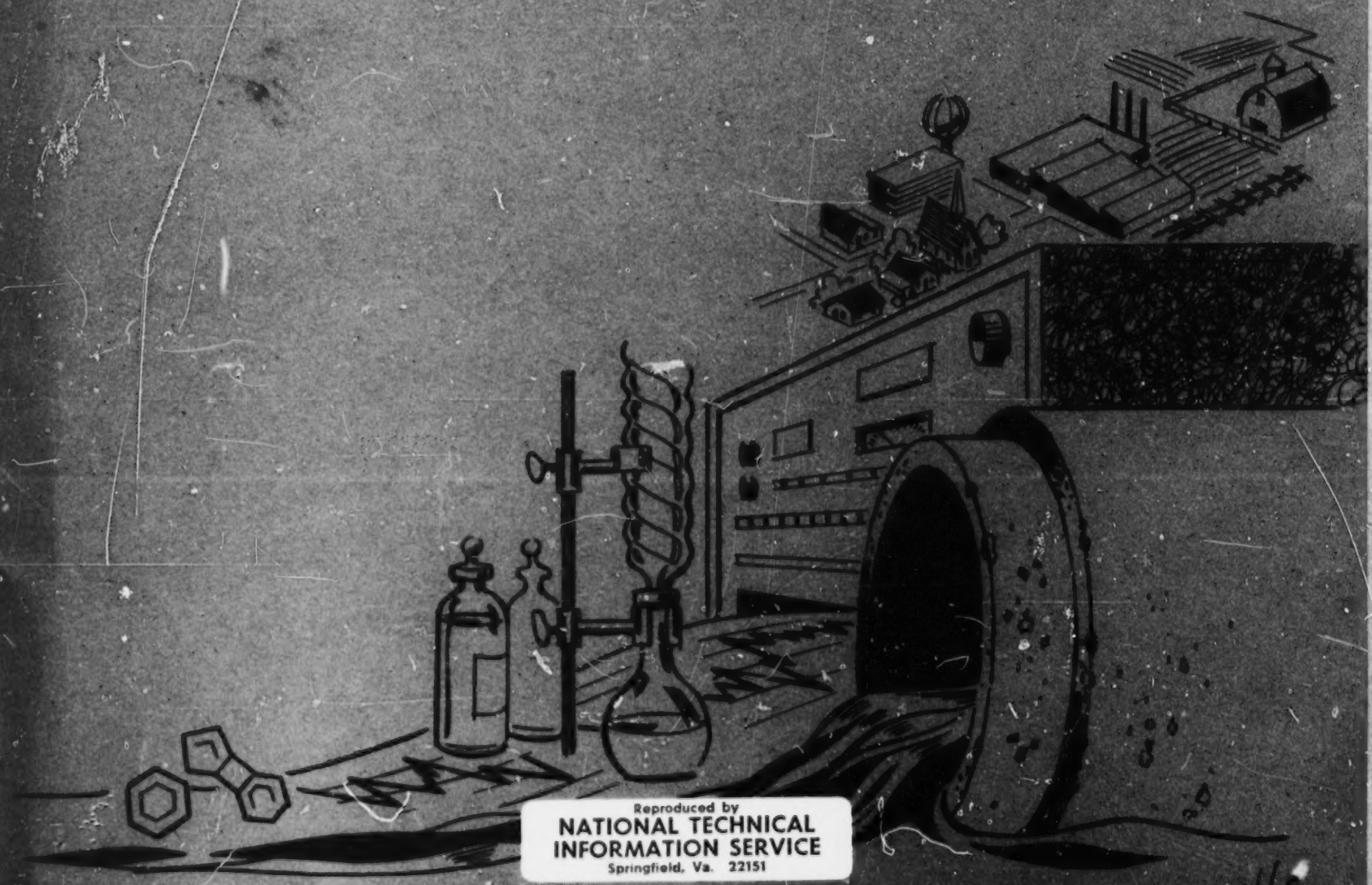
Characterization and Separation of Secondary Effluent Components by Molecular Weight



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1 Accession Number: W72-03784

2 Subject Field & Group: 05A

SELECTED WATER RESOURCES ABSTRACTS
INPUT TRANSACTION FORM

5 Organization: Arthur D. Little, Inc., Cambridge, Mass

6 Title: Characterization and Separation of Secondary Effluent Components by Molecular Weight all Caps

10 Author(s): ~~Levins, Philip L.~~

16 Project Designation: ~~16020 FEN~~ EPA, WQO Contract No. 14-12-886

21 Note: Copy available from GPO Sup Doc as , $0.55 ; microfiche from NTIS as PB 206 175, $0.95.

22 Citation: Environmental Protection Agency Water Pollution Research Series No 16020 FEN, March 1971, 43 p, 10 fig, 5 tab, 2 ref

23 Descriptors (Starred First): *Membrane ~~ultrafiltration,~~ processes, Spectrometers, Municipal wastes, Sewage, *Waste *Chemical analysis, Identification, ~~*High Resolution Mass Spectrometry,~~ ~~Gel Permeation~~ Chromatography, ~~Thin Layer Chromatography,~~ Solvent Extraction, ~~Secondary Waste~~ Effluent, ~~Molecular Weight Determination,~~ Ultrafiltration, Analytical techniques, Separation techniques, Waste water treatment

25 Identifiers (Starred First): Ultrafiltration

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While infrared spectrometry yields good spectra of various effluent fractions, most are still much too complex to be able to infer much specific information from the spectra of the complex mixture. The same is true of ultraviolet spectrometry. High resolution mass spectrometry (HRMS) shows promise for being able to obtain a great deal of specific chemical information even from still very complex mixtures.

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fatty acids, aromatic acids, alkylphenylethylene oxides, chlorinated phenols, aliphatic and aromatic amines, and amides.

(EPA Abstract)

~~This report was submitted in fulfillment of Project Number 16020FEN, Contract 14-12-886, under the sponsorship of the Water Quality Office, Environmental Protection Agency.~~

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WR:102 (REV. JULY 1969) WRSIC

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CHARACTERIZATION AND SEPARATION OF SECONDARY EFFLUENT COMPONENTS BY MOLECULAR WEIGHT

by

Arthur D. Little, Inc.
Cambridge, Massachusetts 02140

for the

ENVIRONMENTAL PROTECTION AGENCY

Project #16020 FEN
Contract #14-12-886

March, 1971

For sale by the Superintendent of Documents, U.S. Government Printing Office, Washington, D.C. 20402 - Price 55 cents

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ABSTRACT

Membrane ultrafiltration with a 1000 MW cut-off membrane is an effective means of separating the high and low molecular weight fractions of effluent and provides useful analytical samples for further study. Solvent extraction techniques and thin layer chromatography show promise as appropriate methods for isolating and resolving the low molecular weight effluent subfraction.

While infrared spectrometry yields good spectra of various effluent fractions, most are still much too complex to be able to infer much specific information from the spectra of the complex mixture. The same is true of ultraviolet spectrometry. High resolution mass spectrometry (HRMS) shows promise for being able to obtain a great deal of specific chemical information even from still very complex mixtures.

Using HRMS the following classes of compounds have been identified in a solvent extract of the low molecular weight effluent fraction:

> fatty acids, aromatic acids, alkylphenyl-
> ethylene oxides, chlorinated phenols,
> aliphatic and aromatic amines, and amides.

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SECTION I

CONCLUSIONS

1. Municipal secondary waste effluent consists of a variety of chemical species ranging from simple molecules to high polymers. Very little is known at the present about the specific chemical composition of these species.

2. Membrane ultrafiltration is an effective means of separating the high and low molecular weight fractions of the effluent for further analytical studies.

3. Solvent fractionation and thin layer chromatographic separation of the low molecular weight fraction provides suitable samples for chemical identification.

4. High resolution mass spectrometry is a very effective means of obtaining a specific identification of the many chemical species present in the isolated analytical fractions.

SECTION II

RECOMMENDATIONS

The purpose of this program was to explore the appropriateness of various methods for separating and characterizing municipal secondary waste effluent. After examining several methods a combination of membrane ultrafiltration, solvent extraction, thin layer chromatography, and high resolution mass spectrometry was found to be most appropriate.

The methodology developed during this study should be applied to a comprehensive study of the low molecular weight effluent species.

Each of the solvent extracts should be separated by thin layer chromatography for chemical identification of the component mixtures by high resolution mass spectrometry. The aqueous phase should also be examined for any remaining organic species.

Derivative formation techniques, such as silylation or acetylation, should be studied for their appropriateness in examining the non-volatile effluent species.

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SECTION III

INTRODUCTION

The overall goal of the program was to develop techniques for separating and characterizing the soluble organic components of secondary effluent from municipal waste treatment plants. The primary emphasis at the outset of the program was on physical means of achieving separation of the sample based on the molecular size and/or weight of the dissolved components. During the course of the study it became apparent in discussions with the project monitor that it would be more appropriate to shift the emphasis to chemical identification of the soluble species, especially the low molecular weight fraction.

To pursue these goals we explored ultrafiltration, gel permeation chromatography, and thin layer chromatography as separation tools and used X-ray and infrared spectrometry for general identification studies and high resolution mass spectrometry for identification of specific chemical species.

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SECTION IV

GENERAL SAMPLE PREPARATION APPROACH

The general procedure developed for sample preparation is shown in Figure 1. The secondary effluent sample was collected from points just before the chlorinator and stored at 4°C prior to filtration. The primary filtration was achieved with a W and R Balston Ltd. 2-micron cellulose filter cartridge held in a Commercial Filters Corporation "Fulflo" water filter. The sample was pressurized in a 5 gallon, stainless steel pressure vessel (Millipore) prior to filtration. This procedure removes most of the large particulate.

Further filtering, plus sterilization by bacteria filtration, was accomplished with a Millipore 0.45 micron filter held in a Millipore Model No. YY30-142-05 holder. Ultrafiltration was then carried out in a Millipore Model No. XX42-142-50 ultrafiltration cell with a series of membranes of different characteristics.

Samples were freeze dried in normal laboratory equipment at various stages for measurement of total dissolved solids and concentration of the sample.

The fractions from the membrane ultrafiltration stage were then studied further by various separation and spectrometric techniques in exploring ways of characterizing the effluent.

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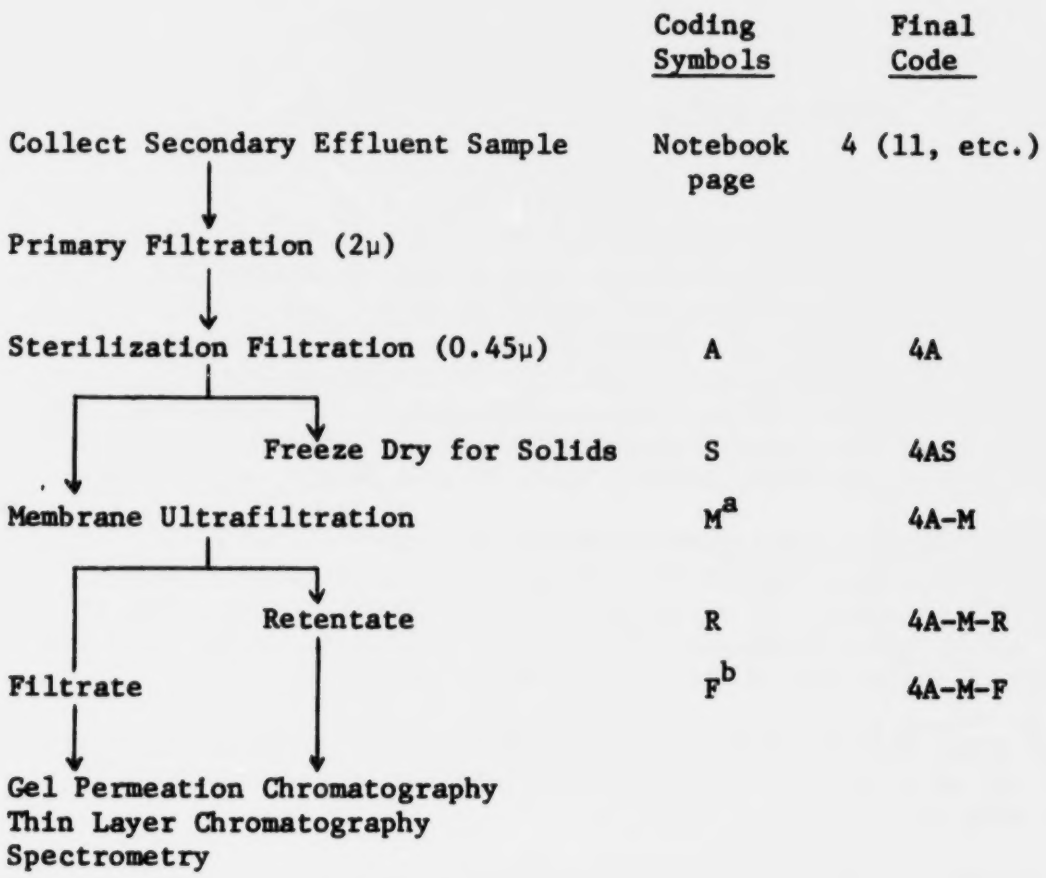


a. Or other appropriate membrane filter code (M = Millipore, A = Amicon, E = Eastman).

b. Sequential fractions numbered thus, F1, F2, etc.

Figure 1. Sample Preparation Procedure

SECTION V

COMPARISON OF THE SOLIDS IN BROCKTON AND ROCKLAND EFFLUENT

Samples of water after secondary waste treatment and before chlorination were obtained from the municipal treatment plants in Brockton, Massachusetts and Rockland, Massachusetts. Both plants operate an activated sludge process. The Brockton facility has a normal process capacity of about 12 mgd with a design limit of 29 mgd, while the Rockland plant presently has a normal load of about 0.8 mgd with a design limit of 2 mgd. The two plants represent a different residential/industrial influx balance and have experienced quite different efflux levels. The two locations, therefore, provided different types of samples for initial evaluation.

Total Solids

In order to characterize the solids content of the samples, the water was removed via freeze drying as follows:

One liter of the sterile liquid was coated on the wall of a 5-liter, single necked, round bottom flask by continuous rotation of the flask until all the liquid was solidified. The flask was then connected by glass tubing to a second 5-liter flask immersed in a dry ice acetone bath.

Then, a vacuum pump was applied and a vacuum of < 1 mm was maintained during the freeze-drying. After the majority of water was removed from the flask (requiring about 24 hours continuous operation), the remaining liquid was transferred into a one-liter flask, and the freeze drying procedure was repeated.

The percent freeze dried solids for each sample was found to be:

-----Sample------		
Source		solids (mg/ℓ)
Brockton	4 AS	280
Rockland	5 AS	360

Ash Content

The solids obtained via the freeze dried process were subjected to an ash analysis at 800°C. The results were:

------ Sample ------		
Source	Code	% Ash*
Brockton	4 AS	71
Rockland	5 AS	61

* percent of solids after water removed via freeze-drying

Infrared Analysis of Freeze-Dried Solids

An infrared spectrum of the total solids (4 AS and 5 AS) showed predominantly water and inorganic salts -- probably sulfates and nitrates. There was only a faint trace of organics (CH_2 bands) indicating that most of the sample was inorganic in nature. The spectrum for sample 4 AS is shown in Figure 2. Therefore, it was decided that to better characterize the organics in these samples, it would be necessary to remove the organic portion by solvent extraction.

A 15 mg portion of solids was extracted sequentially with hexane, chloroform, and methanol using the following procedure:

a) The 15 mg portion was extracted twice (vortex mixer) with 8 ml of hexane.

b) The extract solutions were centrifuged and filtered through Whatman #1 paper.

c) The filtrates were dried under N_2 and the solids mixed with KBr, pressed into a pellet and an IR spectrum obtained.

d) Undissolved solids were rinsed off the filter with chloroform and extracted twice with 5-ml portions of chloroform; steps (b) and (c) were repeated.

e) Remaining solids were then finally extracted with two 8 ml portions of methanol in a similar manner.

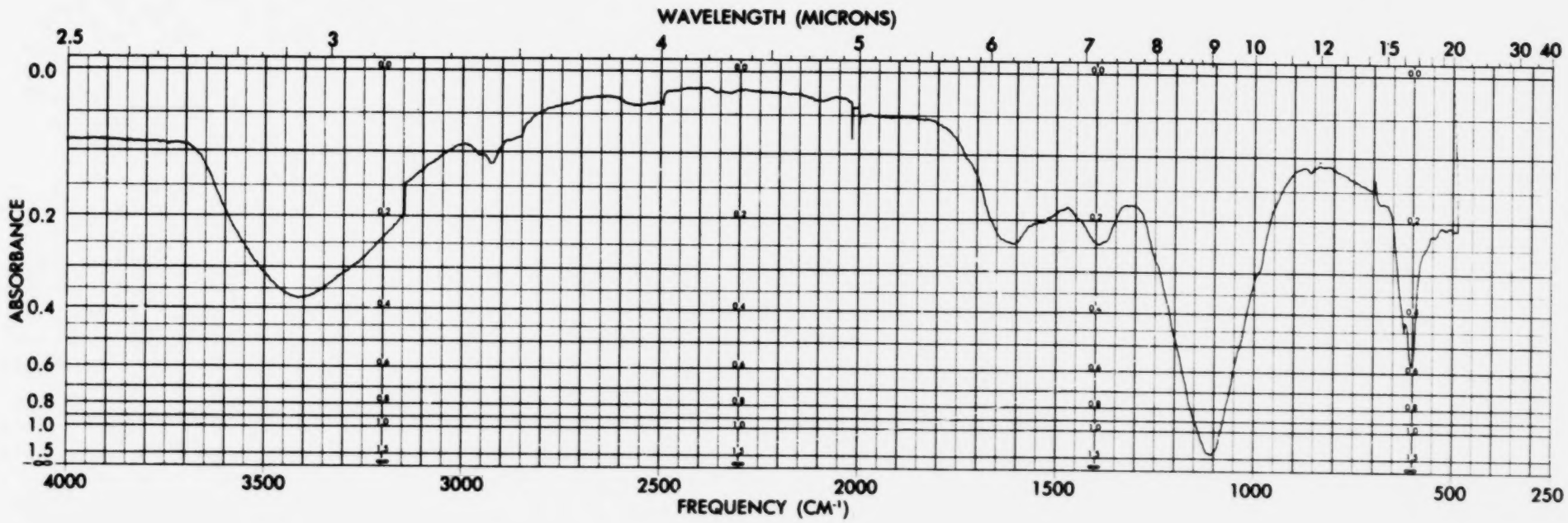


FIGURE 2 INFRARED SPECTRUM OF TOTAL BROCKTON SOLIDS (SAMPLE 4AS)

The results of using this approach are summarized below:

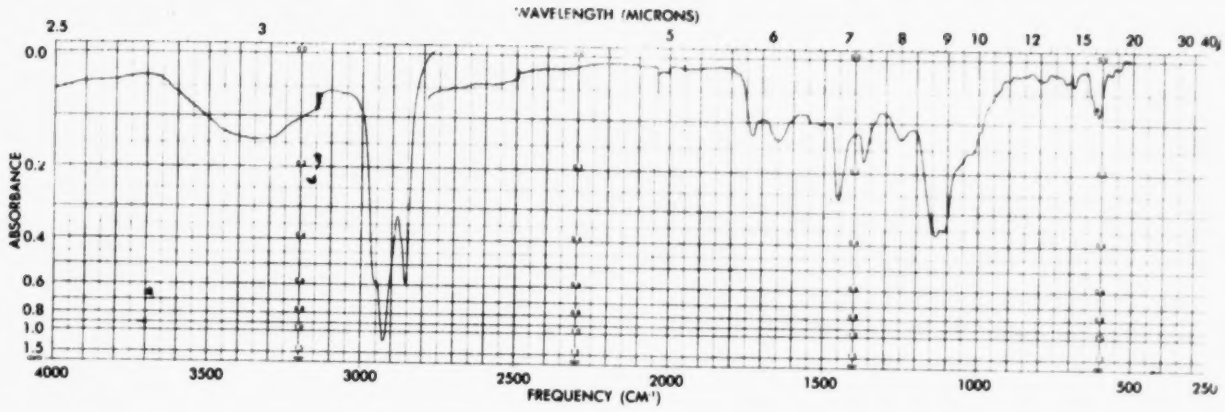
Solids from Brockton Effluent - Sample 4 AS

Hexane extract - approximately 0.05 mg of the original 15 mg of solids (0.3%) were soluble in hexane, and this extract, after removal of solvent, appeared greasy. IR absorptions (Figure 3a) were noted at:

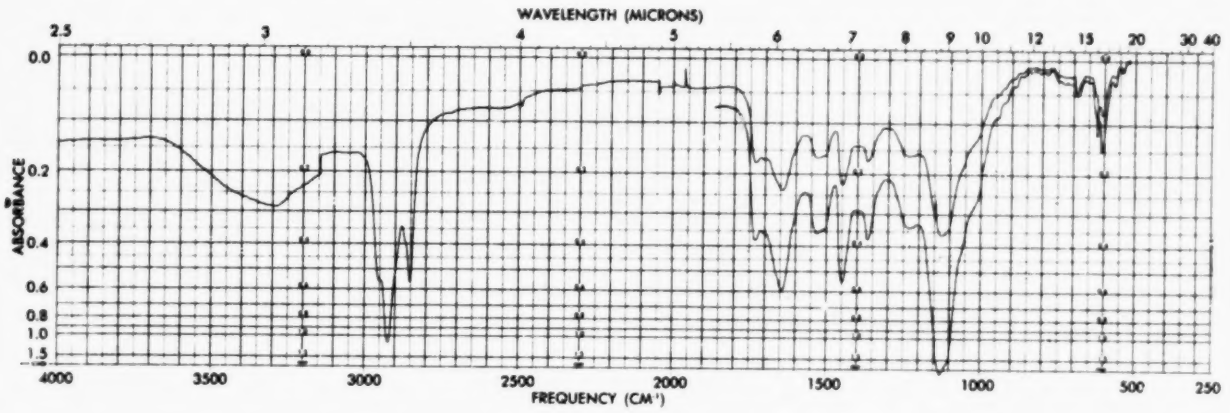
Absorption (cm^{-1})	Intensity	Comment
3350	medium	hydroxyl (acids and alcohols)
2930, 2855, 1465	strong	methylene
2960, 2870, 1380	weak	methyl
1730	medium-weak	ester carbonyl (lipids)
1650	medium-weak	amide carbonyl, or aromatic
1250	weak	carbon-oxygen (C-O)
1140	strong	
610	weak	carbon-halogen

chloroform extract - approximately 0.5 mg (3%) of white, powdery, non-odorous solids were removed. The spectrum (Figure 3b) was very similar to that for the hexane extract except that ester carbonyl was slightly more intense in relation to other bands.

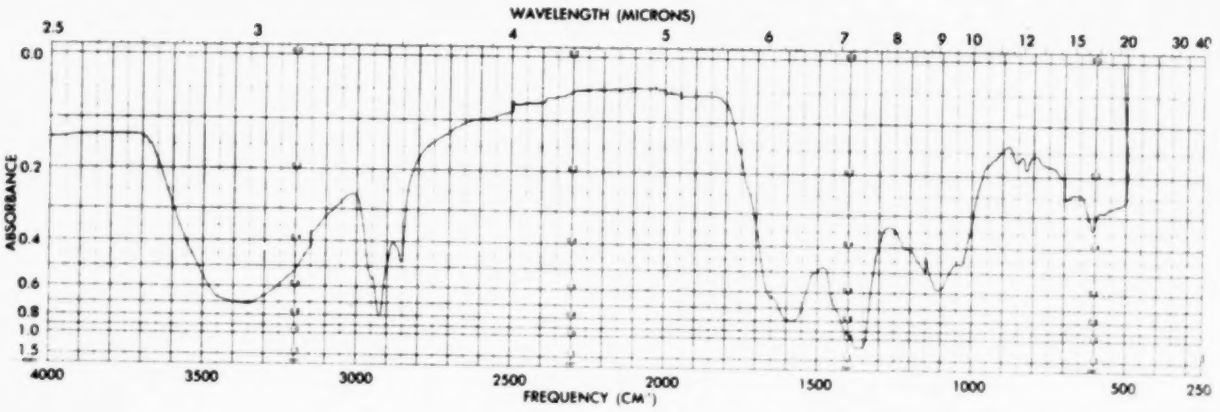
methanol extract - almost 0.5 mg (3%) of material was extracted by the methanol. The resultant solids were pale yellow and waxy in appearance with a sweetish odor. The IR spectrum of this sample (Figure 3c) was different from the other solvent extracts -- notably more hydroxyl, almost no ester carbonyl, and indications of salts (carbonate, sulfate, nitrate). The large hydroxyl band (probably water or methanol) makes meaningful interpretation difficult. It appears that the methanol extracts a large portion of the inorganic fraction suggesting that further extraction should be done only with hexane and chloroform.



A. Hexane



B. Chloroform



C. Methanol

FIGURE 3 INFRARED SPECTRA OF SOLVENT EXTRACTS OF BROCKTON SOLIDS

Solids from Rockland Effluent - Sample 5 AS

The percentage of the original sample which dissolved in each of the three solvents was the same as observed for the Brokton sample. The IR spectra were also the same with the following exceptions:

- The infrared spectrum of the hexane solubles (0.3%) showed little amide (1650 cm^{-1}), much less ester absorption at 100-1150 cm^{-1}, and no C-halogen at 610 cm^{-1}.
- The chloroform solubles (3%) also showed much less ester absorption.

X-Ray Analysis of Freeze Dried Solids

X-ray fluorescence - Both samples (4 AS and 5 AS) showed only the presence of chlorine, sulfur, calcium and potassium. The X-ray technique is capable of detecting all elements of atomic number greater than 13 (Al). Sodium is not easily detected by this technique.

X-ray diffraction - In examining the patterns for the presence of specific crystalline species, both samples showed the presence of sodium chloride. In addition, the freeze dried solids from the Brockton effluent contained potassium chloride, sodium sulfate, and possibly calcium carbonate.

Chemical-Oxygen Demand

Estimates of the chemical-oxygen demand values for these samples and membrane ultrafiltration fractions (discussed in the following section) were also obtained for the purpose of comparing the two samples:

	--COD Value (mg O_2 consumed/liter)--		
Sample Type	Brockton*	Brockton**	Rockland**
0.45 micron filtrate (A)	40	53	72
Ultrafiltration retentate (M-R)	109	133	103
Ultrafiltration filtrate (M-F)	9	-	-

* 1965 APHA Procedure including correction for chloride interference.

** 1955 Procedure without chloride correction.

It is not known at this time how much of the COD value is due to organics and how much is from inorganics. The data show the two sources of sample to be similar in terms of oxygen demand.

Conclusions

The conclusions reached from this portion of the study were that the soluble portion of the water samples from both waste treatment plants are predominantly inorganic. This is in accord with other published results[1] where organics averaged 55 mg/ℓ with a total dissolved solids being 730 mg/ℓ.

By the procedures described above we have found the bulk of the inorganics to be sodium, calcium, carbonate, chloride, and sulfate with lesser amounts of potassium, ammonium, magnesium, nitrate, silica, and phosphate. Our data did not suggest any particular differences in the two sources of the samples, and we chose to pursue our studies with samples of the Brockton effluent primarily because of access convenience. The solubility/IR spectral data from the solids also suggested that the greatest amount of organic chemical detail could be obtained from studies of the hexane/chloroform extracts.

SECTION VI

SEPARATION BASED ON MOLECULAR SIZE

Several studies have shown that secondary effluent consists of organic materials ranging from low molecular weight to natural polymers. Separation techniques were examined to determine whether classification by effective molecular size could be a useful characterization technique and could also provide samples useful in the successive analysis steps.

ULTRAFILTRATION

Several membranes were evaluated for their effectiveness in modifying the filtered effluent samples in a manner which would be most useful for further characterization studies. Since most specific chemical analysis techniques tend to have a cutoff in the 500-1000 MW range, it seemed most appropriate to study those membranes which could separate the sample based on effective molecular size at about MW 1000. The Millipore PSAC 142-05 (M), Amicon UM-2 (A), and Eastman HT-00 (E) membranes were examined. All of these membranes were reported to have nominal 1000 MW cut-offs.

The Millipore filter is made from mixed cellulose esters, while the Amicon UM-2 is a polyelectrolyte complex which can have ion exchange properties. The Eastman membrane is an open Loeb cellulose acetate type (wet gel) with low salt rejection. These three filters were selected because they have different properties, and the samples could be separated in different ways.

Before use, each membrane was washed with sterilized distilled water to remove any residual trace constituents left during the manufacturing process and to obtain some measure of the membrane's performance. The latter was accomplished by observing the water flux (flow per unit time) at 50 psi, an indirect measure of pore size and distribution.

The primary means of judging the effect of the ultrafiltration on the effluent samples was by comparison of the gel permeation chromatogram (GPC) of the filtrate and the retentate obtained after filtering with each of these membranes. The details of the GPC results are given in the following section.

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Millipore PSAC 142-05

Samples 4A and 11A of the Brockton effluent were subjected to ultrafiltration on the Millipore membrane. A 700 ml aliquot of effluent was placed in the cell and 480 ml were forced through the membrane (requiring 50 psi nitrogen). The resultant samples were characterized by chemical-oxygen demand, gel permeation chromatography and ultraviolet spectrophotometry. Unfortunately, the UV analyses were interfered with seriously, because it was found that the membrane bleeds a UV absorber unless washed with large amounts of water.

The GPC tracings (Figures 6-9) obtained on these samples gave some indication of a molecular size separation, but the contamination problems associated with the membrane suggested that it was generally as useful for the purposes of the program as the Eastman membrane. The COD values reported earlier indicate that most of the oxidizable sample was retained by the membrane.

Amicon UM-2

Our efforts to employ the Amicon membrane were unsuccessful due to blistering of the membrane in the Millipore separator system. We have not resolved whether this is a typical problem with this combination or only a bad lot of membranes. Due to the change in emphasis during the program, we did not pursue this matter.

Eastman HT-00

The Eastman membrane was placed in the Millipore cell and washed with distilled water until the filtrate showed no absorbance at 250 mμ in a 1 cm cell. Then the cell was charged with 1000 ml of Brockton sample. The cell was pressurized to 100 psi with nitrogen and water forced through the membrane. The first 50 ml of filtrate were collected and coded F1. Successive fractions of 250 ml, 200 ml and 200 ml were collected and coded F2, F3, and F4. Then the cell was depressurized, an additional 700 ml of water added, and the latter forced through the membrane. This filtrate was coded FW1.

The GPC tracings for the initial solution, retentate and filtrate are shown in Figure 10. It is evident from these results that the Eastman membrane is effectively separating the sample into two fractions, presumably on the basis of molecular size, and this membrane was chosen for sample preparation for the remainder of the project. Several samples of filtrate and retentate were obtained by filtering approximately 800 ml samples of Brockton sample 11A with the Eastman membrane in the Millipore ultrafiltration apparatus. Resulting fractions are coded 11A-E-R or 11A-E-F for retentate and filtrate.

Solids were isolated from the membrane fractions using the freeze drying technique. Solids content and ash contents of the solids were as follows:

Sample	Solids Content	Ash Content of solids (%)
11AS (S for solids)	240 mg/ℓ	
11A-E-RS (retentate)	370 mg/ℓ	50.0
11A-E-FS (filtrate)	180 mg/ℓ	44.7

With the samples corrected to original volume -- based on the approximately 75% of sample volume which passed through the membrane -- (90 mg/ℓ for RS and 140 mg/ℓ for FS), the data suggest that approximately 61% of the solids pass through the Eastman filter. The retentate and filtrate solids do not differ appreciably in ash content.

A sequential solvent extraction of the solids revealed the following solubilities:

	--% Extracted--	
Solvent	RS	FS
hexane	3	1
chloroform	1	trace
methanol	26	49

An examination of the infrared spectra of the total solids and the extractable portions, while suggesting small amounts of organic species, was not especially useful in identifying classes of organic compounds, X-ray fluorescence examination of the RS and FS samples revealed the normally observed presence of calcium, chlorine, sulfur and potassium with possible traces of phosphorus, iron and copper.

GEL PERMEATION CHROMATOGRAPHY (GPC)

Several studies were carried out to examine the usefulness of GPC both as a means of sample characterization itself and as a means of further sample separation to aid the chemical identification efforts.

A number of columns have been evaluated, including those prepared from Sephadex (a polycarbohydrate), BioGel (a polyamide) and Corning CPG (porous glass). Nominal molecular weight cutoffs range from 700 to 30,000 (dextrose). We recognized in initiating these studies that GPC behavior in aqueous systems is not well understood at the present time. High concentrations of salts will affect the elution volume of organics, and the variety of charged species in an effluent sample will interact in different ways with the polar gel sites. The GPC studies were also used partly to evaluate the effect of the membrane filtration.

The GPC studies reported were performed using the appratus shown schematically in Figure 4. The solvent was water, with flow rates ranging from 0.2 to 0.5 ml/min. Sample size injected into the column has ranged from 20 µℓ to 200 µℓ. A variety of columns and samples have been studied, the results of which are given below:

Sephadex G-10 (Exclusion limit of 700)

Some preliminary experiments were run using a Sephadex column with a low exclusion limit (nominal molecular weight of 700). For this we compared an inorganic salt (NaCl) with the soluble portion of the total solids recovered by freeze drying the 0.45 micron filtered waste water (Sample 11 AS). Figure 5 shows the GPC curves for these samples using a Laboratory Data Control refractive index detector. It can be seen that a separation of the soluble filtrate solids has occurred. However, it also indicates that more adequate differentiation might be obtained on a column with a higher exclusion limit.

Sephadex G-25 (Exclusion limit of 5,000)

A comparison of the GPC behavior of Brockton retentate and filtrate on Sephadex G-25 is shown in Figure 6 (ultraviolet absorption detector). From this, it can be seen that the Millipore pellicon ultra filter (nominal MW cutoff of 1,000) is separating the waste water sample into two distinctive fractions -- the filtrate carrying mostly low molecular weight material, while the retentate has a wider spectrum of material.

The patterns for a fresher sample of Brockton water (11a), before and after ultrafiltration (Millipore), are given in Figure 7. This series of curves was obtained the same day and is useful for comparison of relative amounts in each fraction. The large difference in appearance between the results reporesented in Figures 6 and 7 is unknown but could be due to differences in initial sample composition.

BioGel P-30 (Exclusion limit of 30,000)

Figure 8 presents GPC curves for sodium chloride and two samples on BioGel P-30. Once again, a number of peaks are evident in the effluent samples. The freeze-dried filtrate (Curve c) shows better resolution here than in Figure 5 on Sephadex G-10 (the detector difference could influence this comparison) -- something to be expected if larger molecules are present. The curve for the retentate (Curve b) is very similar to the one for that sample on Sephadex G-25 (Figure 7).

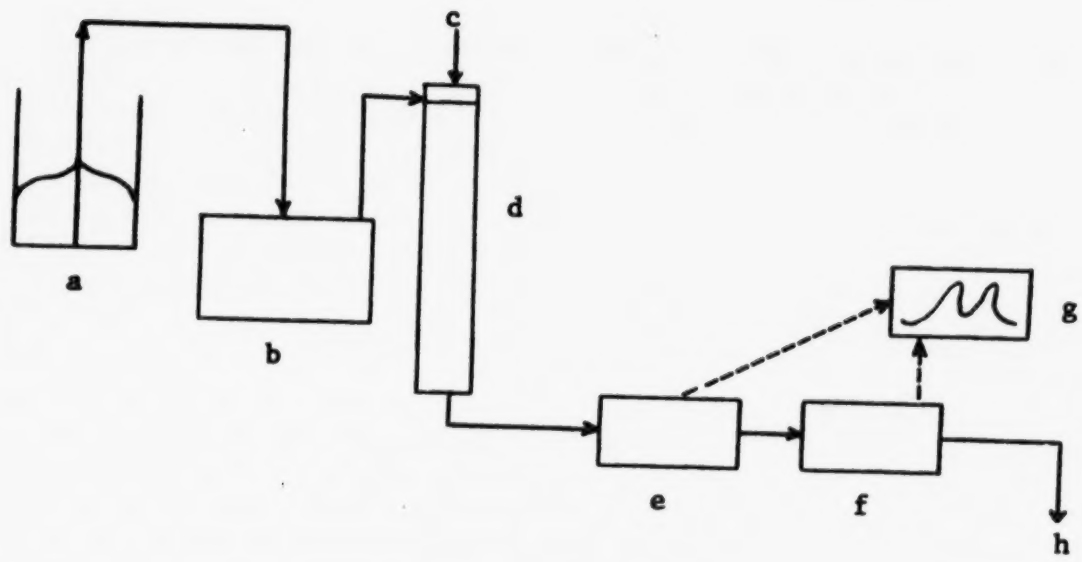
Corning Glass CPG-10 (75A^{o} limit of 28,000)

The column prepared from the particular porous glass studied did not demonstrate much distinctive separation for the retentate sample (Figure 9). This is a result similar to that observed in some preliminary work done at Waters Associates.

GPC Studies of Eastman Membrane Samples

In order to compare the efficiency of the Eastman (E) and Millipore (M) membranes in the ultrafiltration process, the original sample and the ultrafiltration retentate and filtrate were examined on a Sephadex column. The column actually consisted of three, three-foot sections in series containing Sephadex G-10, G-15, and G-25 successively; these have nominal high molecular weight cutoffs at 750, 2500 and 5000 respectively. A comparison of these curves in Figure 10 with the Sephadex curves in Figures 6 and 7 using the samples from the Millipore membrane suggest that the Eastman membrane gives a more distinct separation between the retentate and filtrate and would, therefore, be more useful for preparing analytical samples.

The net conclusion of these GPC studies is that it does appear possible to achieve some resolution and characterization of the samples using this approach. However, as a characterization aid there does not appear to be enough detailed information to be truly useful, and there does not appear to be any really significant separation advantages to the approach as an analytical aid. It appears that the membrane ultrafiltration step alone gives the greatest assistance in terms of sample fractionation.



a. Solvent Reservoir - Glass bottle

b. Pump - Milton Roy, Milroyal, capacity 123 ml/hr

c. Sample Injector - Septum and syringe

d. Column - 1/4" x 3' stainless steel

e. Refractive Index Detector - Laboratory Data Control UV monitor, peak wave length @ 254 mμ, 8 1 flow-through cell

f. Refractive Index Detector - Laboratory Data Control refractomonitor (temperature stabilized 25°C by water from a Hoake circulating bath) flow-through cell with a volume of 3 μl

g. Recorder - Hewlett-Packard, Moseley 7100B - dual channel recorder

h. Fraction collector or discard

Figure 4: Schematic Representation of GPC System

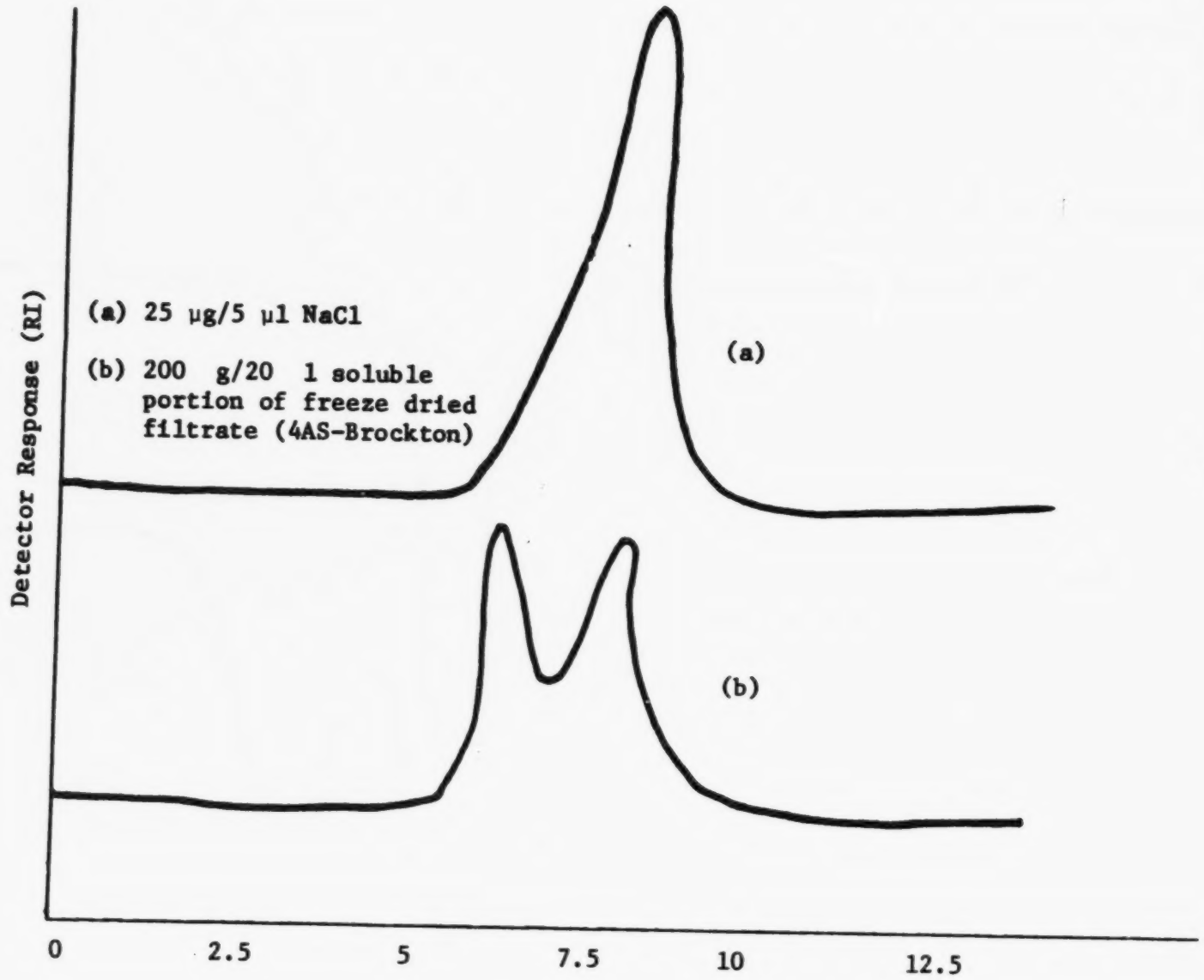


Figure 5: GPC Curves on Sephadex G-10

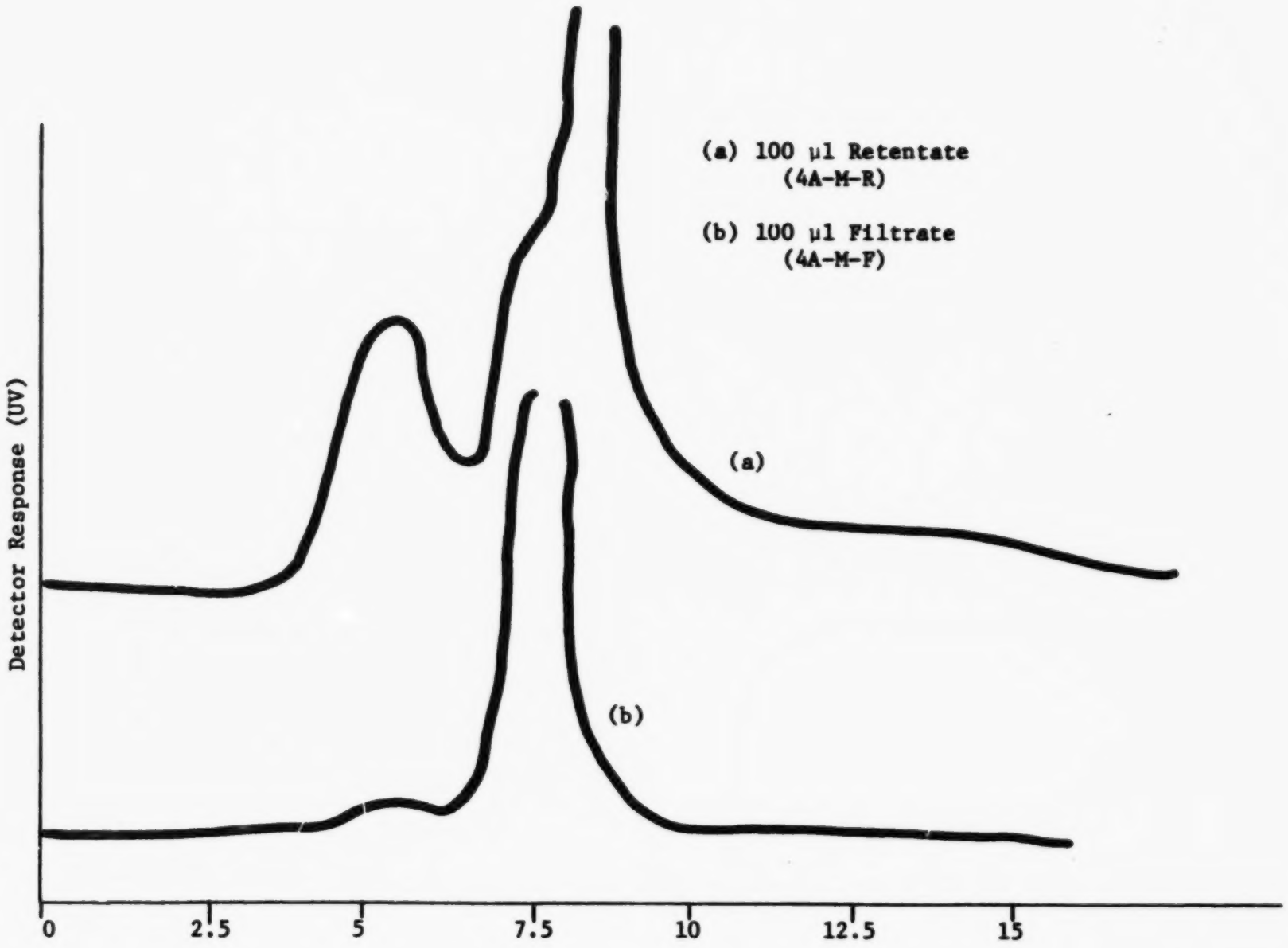


Figure 6. GPC Curves on Sephadex G-25 of Brockton Effluent After Ultrafiltration (Millipore)

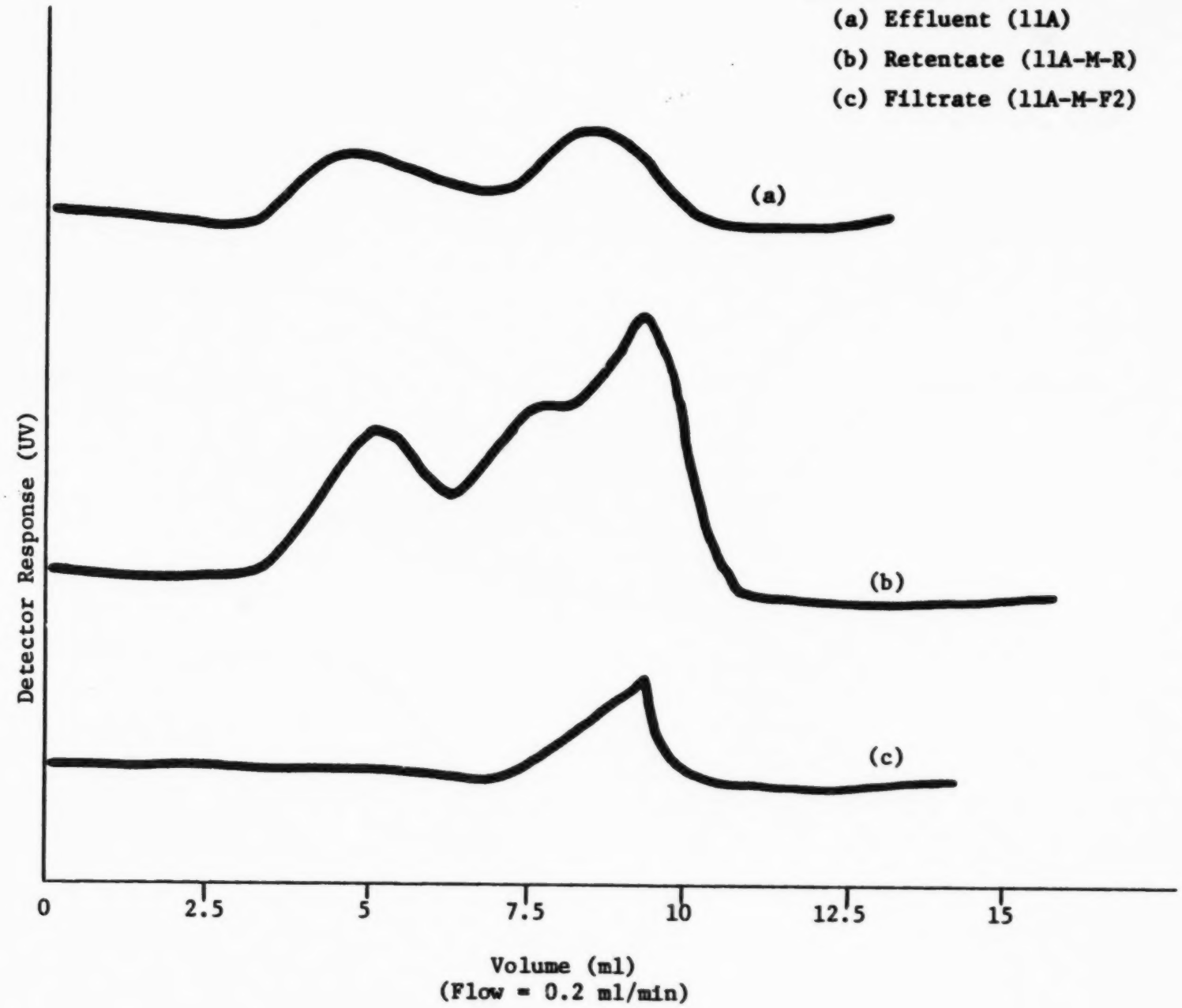


Figure 7. Brockton Sample 11A -- GPC Curves on Sephadex G-25

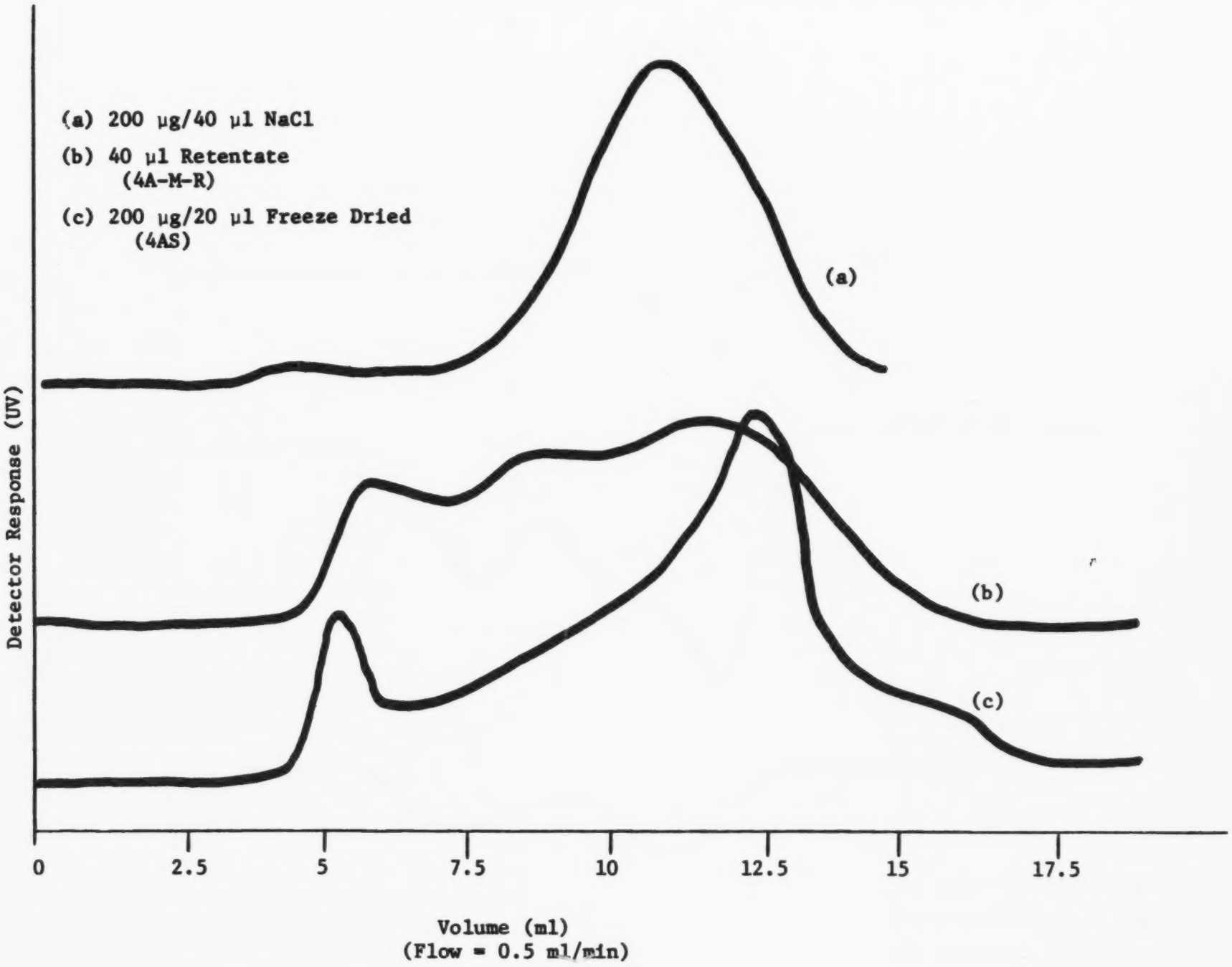


Figure 8. GPC Curves on BioGel P-30 of Brockton Sample 4A

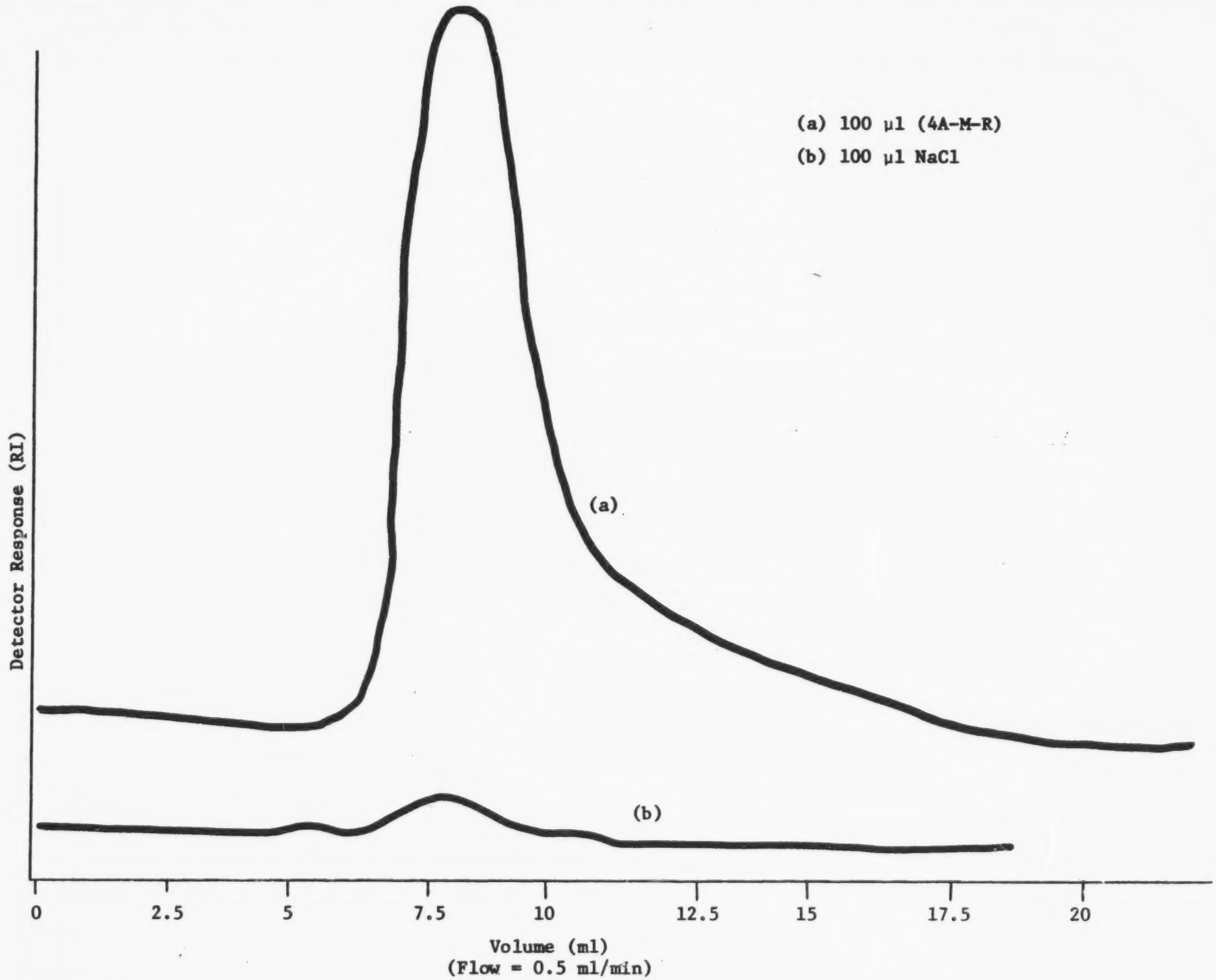


Figure 9. GPC Curves on Brockton Sample on Corning-Glass CPG-10

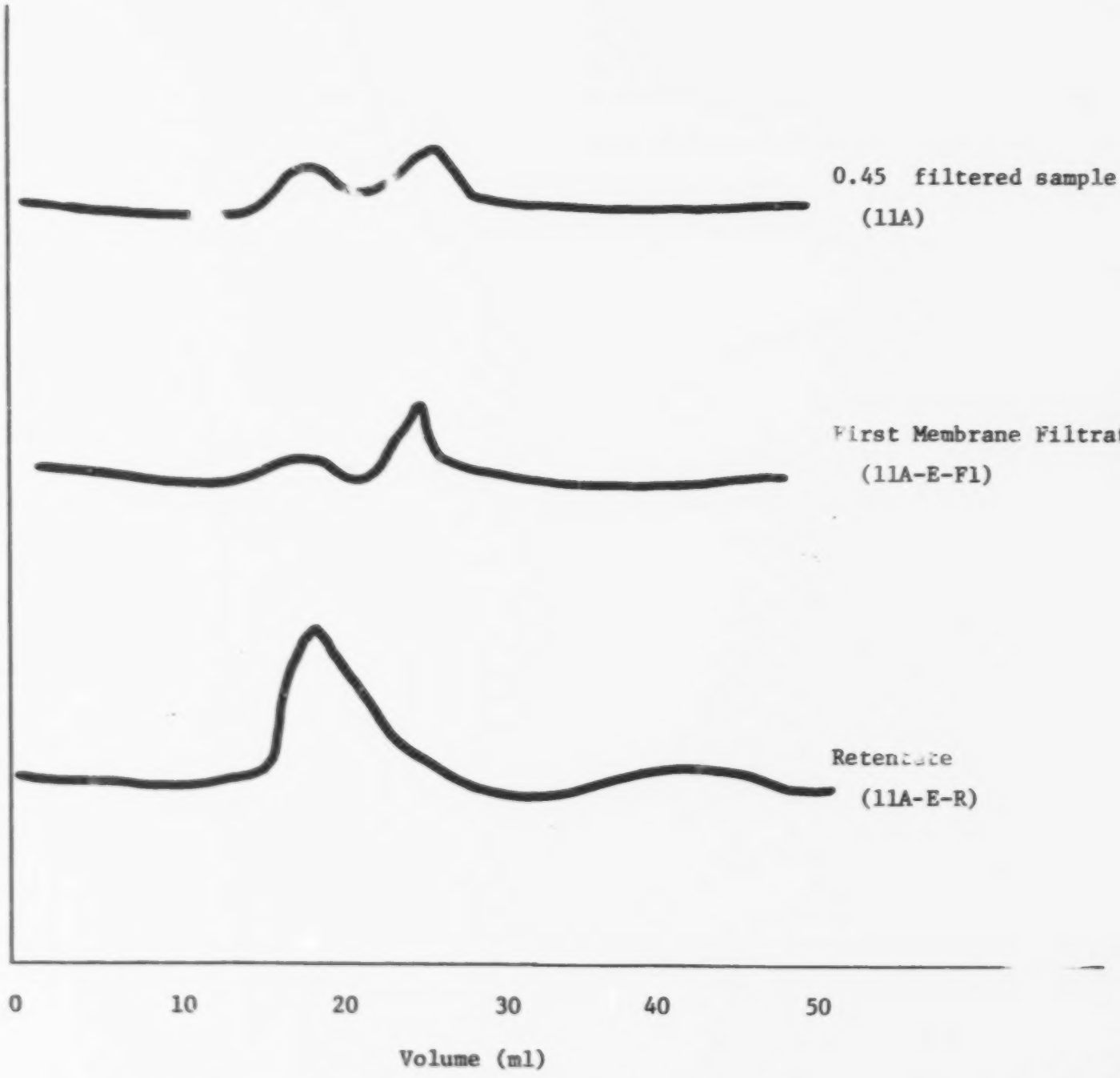


Figure 10. GPC Curves for Brockton Effluent from Eastman Membrane Ultrafiltration

SECTION VII

SOLVENT EXTRACTION

In our experience with previous problems of this sort[2] dealing with dilute mixtures of a large variety of structure types, we have found that extraction of the aqueous phase with a non-polar solvent such as hexane and a polar solvent such as chloroform achieves a nearly complete extraction of all non-polymeric organic materials, except possibly the very small molecular weight acids and alcohols, and provides an efficient fractionation of the sample on the basis of structure polarity. We have also found that species such as acids and phenols may be present as salts and require acidification before they can be extracted into t the organic solvents.

Accordingly, the Brockton effluent samples (11A) were extracted first with hexane and then with chloroform, acidified to a pH of 1-2 and extracted a second time with each of the two solvents. The procedure is shown schematically in Figure 11 for the filtrate fraction. The same procedure was used for the retentate. The infrared and ultraviolet absorption spectra of each of the extracts were examined for evidence of structural features. A brief summary of the observations from these samples is given in Table 1.

No clear differences were observed in the spectra of the extracts of the as-is and acidified samples, although we expected some change since the original pH was about 6-7.

It is difficult to satisfactorily interpret the significance of a composite spectrum of such a complex sample. However, the infrared spectra do indicate several major functional groups. Some of the observed hydrocarbon absorption bands observed in the hexane extracts may be due to impurities later found to be present in the hexane solvents. The infrared spectra on the CHLOR extract indicate the presence of an alkylated phenyl-ethylene oxide adduct of the type used as a base for detergents, cleaning agents or emulsifiers.

Successful comparison has been made of spectra of known compounds such as Tergitol, Igepal, and Hostapol. It could have its origin in some of the industrial makeup of the treatment plant intake.

The ultraviolet absorption maxima indicate generally the presence of aromatic or conjugated species in each of the fractions, including some with fairly extensive chromophores extending the absorption out to 340 nm.

The lower molecular weight filtrate fractions were each studied by hi-' resolution mass spectrometry as reported later.

SOLVENT EXTRACTION PROCEDURE

Sample 11A-XE-F

(2 800 ml samples of Eastman HTOO filtrate)

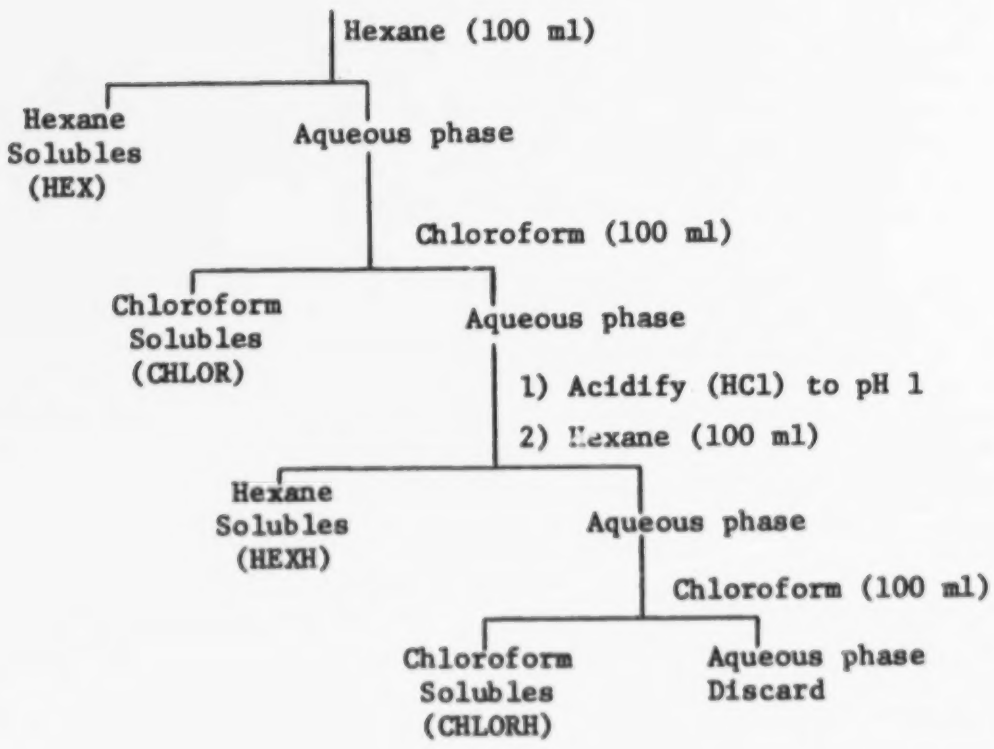


Figure 11. Solvent Extraction Procedure

TABLE 1

Infrared and Ultraviolet Spectra of Solvent Extractable Organics

			Spectral Features	
Sample	Treatment	Extracting Solvent	Infrared	Ultraviolet (maxima nm)
Filtrate	as is	hexane	hydrocarbon	340, 325, 280, 270, 247
Filtrate	as is	chloroform	carbonyl, unsaturation	270
Filtrate	acidified	hexane	hydrocarbon	270
Filtrate	acidified	chloroform	carbonyl, unsaturation	275
Retentate	as is	hexane	hydrocarbon, carbonyl	288, 260, 245
Retentate	as is	chloroform	hydrocarbon, carbonyl	298, 260
Retentate	acidified	hexane	hydrocarbon	265, 260, 230
Retentate	acidified	chloroform	acid	(insufficient sample)

SECTION VIII

THIN LAYER CHROMATOGRAPHY (TLC)

Since most of the more polar and reactive species were likely to be present in the chloroform extract, we explored the suitability of thin layer chromatography (TLC) for resolution of this extract prior to identification by mass spectrometry.

Behavior of the acidified filtrate chloroform extract (CHLORH) was examined on Silica Gel HF using ultraviolet absorption and iodine detection to evaluate the separation. Mixtures of chloroform and methanol were evaluated as eluting solvents including 100% chloroform as well as 5, 7.5 and 10% methanol in chloroform.

The 10% methanol system had the best overall behavior and gave the TLC observations reported in Table 2. The spots were extracted with a 20% methanol in chloroform solution, while the origin was extracted with a 50% methanol solution. The presence of amines in this extract was indicated by the positive response to ninhydrin reagent in some of the TLC tests. Each of the coded sample areas was isolated for study by high resolution mass spectrometry.

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TABLE 2

TLC Behavior of CHLORH Fraction

----Eluting Areas*------		
Code for HRMS	R_f	Ultraviolet Maxima (nm)
TLC 6	0.8 (major)	258
TLC 5	0.7 (major)	255
TLC 4	0.5	-
TLC 3	0.4	260
TLC 2	0.3	255
TLC 1	0.25	-
-	origin (major)	270

*Adsorbent - Silica Gel HF

Solvent - 10% Methanol/90% Chloroform

SECTION IX

HIGH RESOLUTION MASS SPECTROMETRY (HRMS) OF LOW MOLECULAR WEIGHT FRACTIONS

High resolution mass spectrometry (HRMS) studies have been completed on the solvent extracts of the membrane filtrate and also on the regions separated from the acidified chloroform extract by thin layer chromatography (TLC), obtained as described in the last two sections. The samples examined at this stage have proven to still be extremely complex mixtures and detailed chemical structure assignments can only be made on a tentative basis. The inherent resolving power in the HRMS technique was able, however, to demonstrate the power of using this approach in characterizing such samples.

Experimental

Attempts were first made to obtain HRMS on the four solvent extracts by drying (in a stream of nitrogen) an aliquot in a small glass container and placing the dried sample in the glass reservoir inlet of the spectrometer, where the vaporization temperature is 200°C. No volatile species were observed in any of the samples in this manner, indicating the lack of low molecular weight volatile species in the samples. This observation could be the result of our sample preparation procedures, but further studies would be needed to check this point.

Spectra were finally obtained by drying portions of the solvent extracts and extracted TLC regions in small glass melting point capillaries and placing these in the direct insertion probe. The probe was then positioned in the cooled (about 80°C) spectrometer source where high resolution spectra were recorded on photoplates as the temperature of the source was gradually increased to a limit of about 250°C. After examining the photoplates, we concluded that all of the information from each sample could be obtained by processing the spectra obtained about 100°C and 200°C. No additional information was observed at the other temperatures. Spectra were also obtained on hexane and chloroform solvent blanks.

The photoplate spectra were processed under digital computer (Hewlett-Packard (2116B) control on a Grant comparator-microdensitometer. The basic digitized peak profile data were stored on magnetic tape and later processed for spectral output on an IBM 360/65.

RESULTS

The output from each spectrum represented a still highly complex mixture with typical results showing 2-6 different specific chemical compositions at each nominal mass value. We have extracted from these data the observations that represent the major species present in the samples and the species for which some tentative assignments could be made.

It is important to remember in interpreting complex spectra such as from these samples, that one can only make initial estimates as to the structure types represented by the computed elemental compositions. More detailed sample resolution will be required before the structures for most of the species can be assigned.

The results from the solvent extracts are given in Tables 3 and 4, and the results from the TLC separated regions are in Table 5. The data represent a summary of the results from the spectra of the samples at the two temperatures. Large amounts of both aliphatic and aromatic hydrocarbon were observed in all of the samples.

Unfortunately, the background interference from the hexane solvent blank was sufficiently high that we were unable to determine whether or not any hydrocarbon species could be attributed to the sewage effluent, and these data have been eliminated in the tables. The chloroform blank had only a small amount of hydrocarbon background.

The data in Tables 3 to 5 represent only a small amount of the data potentially available from the effluent samples. It serves best to demonstrate the type of information which may be gained. While most structure assignments are very tentative, the evidence is quite good for a few specific choices such as the aromatic ethyleneoxide, nicotine, and fatty acids. Carbohydrates and amino acids would not have been observed with the approach used because of their lack of volatility. Lipids (triglycerides) would have been observed but appear not to have been present in the samples.

Many aromatic amines were observed and a few aliphatic ones. Some of the species containing nitrogen and oxygen may be amides. An alkylated phenyl ethylene oxide, previously identified from the infrared spectra, is frequently present in many of the fractions. Its high concentration may be due to its use as an industrial wetting agent. The interesting series of chlorinated phenols may also have their origin in industrial use.

In summarizing these findings, the chemical classes tentatively identified as being present in the membrane filtered effluent include:

> fatty acids, aromatic acids, alkylphenylethylene oxides, chlorinated phenols, aliphatic and aromatic amines, amides, and several still unidentified oxygen and nitrogeneous species.

The samples do not appear to contain low molecular weight volatile species, and no lipids were observed. A large hydrocarbon background interference problem resulted in not being able to assess the presence of this class in the samples. Of course, the species still present in the aqueous phase have not been examined in this analysis, but our experience has been that those chemicals amenable to HRMS studies would have been observed in the work reported. Those still present in the aqueous phase usually are not volatile enough for study. Through suitable derivative formation, however, it may be possible to study additional parts of the sample.

The preliminary results, represented in these samples, clearly demonstrate that the HRMS technique is a powerful means of obtaining specific chemical information on complex samples. The potential of the method is still comprised, however, by the greatest number of species present in the samples. We recommend that in future programs an approach be developed for identifying specific compounds based on the application of column, thin layer, and gas chromatography to obtain sample resolution, followed by identification using high resolution mass spectrometry.

TABLE 3

Summary of HRMS Results from Solvent Extracts of As-Is Sample

Extract - HEX*

MW	Elemental Composition	Possible Structure (or type)
228	$C_{14}H_{28}O_2$	fatty acid
240	$C_{16}H_{20}N_2$	aromatic amine
203	$C_{15}H_9N$	aromatic amine
193	$C_{14}H_{11}N$	methyl quinoline
179	$C_{13}H_9N$	quinoline
183	$C_{13}H_{13}N$	aromatic amine
147	$C_8H_5NO_2$	phthalimide (?)
121	$C_8H_{11}N$	alkyl aniline

Extract - CHLOR*

438	$C_{29}H_{26}O_4$	? (379-$C_{26}H_{19}O_3$ fragmentation
256	$C_{16}H_{32}O_2$	fatty acid
176	$C_{10}H_{12}N_2O$	? (aromatic)
121	$C_8H_{11}N$	alkyl aniline
98	C_5H_8NO	? possible amide fragmentation

* both samples have series of oxygenated peaks consistent with an alkylated phenylethylene oxide.

TABLE 4

Summary of HRMS Results from Solvent Extracts of Acidified Sample

Extract - HEXH*

MW	Elemental Composition	Possible Structure (or type)
264	C_6Cl_5HO	pentachlorophenol
230	$C_6Cl_4H_2O$	tetrachlorophenol
196	$C_6Cl_3H_3O$	trichlorophenol
146	$C_6H_4Cl_2$	dichlorobenzene

Extract-CHLORH*

284	$C_{18}H_{36}O_2$	fatty acid
256	$C_{16}H_{32}O_2$	fatty acid
236	$C_{16}H_{28}O$	?
170	$C_{12}H_{10}O$	diphenyl ether (?)

* both samples have series of oxygenated peaks consistent with an alkylated phenylethylene oxide.

TABLE 5

HRMS Results on TLC Separated CHLORH Sample

MW	Elemental Composition	Possible Structure (or type)
TLC-1		
very weak spectrum - traces of aliphatic acids		
TLC-2		
176	$C_{10}H_{12}N_2O$	? (aromatic)
164	$C_9H_{12}N_2O$	? (aromatic)
157	$C_{11}H_{11}N$	dimethyl quinoline
143	$C_{10}H_9N$	methyl quinoline
101	$C_6H_{15}N$	aliphatic amine
TLC-3		
very weak spectrum - traces of oxygenated species		
TLC-4		
aliphatic acid fragment ions from C_6-C_{10}		
162	$C_{10}H_{14}N_2$	nicotine
93	C_6H_7N	aniline
TLC-5		
284	$C_{18}H_{36}O_2$	fatty acid
256	$C_{16}H_{36}O_2$	fatty acid
148	$C_{10}H_{12}O$	?
bromine fragment ions at 79/81(Br), 80/82(HBr) indicating presence of brominated species		
TLC-6		
267	$C_{15}H_{23}O_4$	major component. These are all fragment ions from an alkylated phenyl-ethyleneoxide.
205	$C_{13}H_{17}O_2$	
177	$C_8H_{17}O_4$	
161	$C_{11}H_{13}O$	
135	$C_9H_{11}O$	
133	$C_6H_{13}O_3$	
311	$C_{20}H_{25}NO_2$	? (aromatic)
176	$C_{10}H_{12}N_2O$	? (aromatic)
175	$C_{11}H_{13}NO$	
142	$C_9H_{20}N$	aliphatic amine fragmentation
170	$C_{12}H_{10}O$	diphenylether

SECTION X

ACKNOWLEDGMENTS

The cooperation and assistance of Mr. Timothy Murphy, Supervisor of the City of Brockton Water Pollution Control Facilities is sincerely acknowledged.

We wish to thank Dr. Aaron A. Rosen and Dr. Barry M. Austern, the Project Officer of the Water Quality Office, Environmental Protection Agency for their guidance in providing direction of the program, and we also wish to thank the Office for its support of this project.

The laboratory studies were conducted by Mr. Clifford H. Summers, Miss Ann L. Hynes and Mr. Arthur A. Massucco. Drs. John T. Funkhouser and James E. Oberholtzer contributed to design of the laboratory experiments and interpretation of the data.

SECTION XI

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2. "Chemical Identification of the Odor Components in Diesel Engine Exhaust", final report by Arthur D. Little, Inc. on APRAC Project No. CAPE-7-68 to Coordinating Research Council and Environmental Protection Agency, June 1970. NTIS* Accession No. PB 194-144.

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1 Accession Number	2 Subject Field & Group	SELECTED WATER RESOURCES ABSTRACTS INPUT TRANSACTION FORM
W	05A	

5 Organization

Arthur D. Little, Inc.

6 Title

Characterization and Separation of Secondary Effluent Components by Molecular Weight

10 Author(s)

Levins, Philip L.

16 Project Designation

16020 FEN
EPA, WQO Contract No. 14-12-886

21 Note

22 Citation

23 Descriptors (Starred First)

*Membrane ultrafiltration, *High Resolution Mass Spectrometry, *Chemical Identification, Gel Permeation Chromatography, Thin Layer Chromatography, Solvent Extraction, Secondary Waste Effluent, Molecular Weight Determination

25 Identifiers (Starred First)

27 Abstract Membrane ultrafiltration with a 1000 MW cutoff membrane is an effective means of separating the high and low molecular weight fractions of effluent and provides useful analytical samples for further study. Solvent extraction techniques and thin layer chromatography show promise as appropriate methods for isolating and resolving the low molecular weight effluent subfraction.

While infrared spectrometry yields good spectra of various effluent fractions, most are still much too complex to be able to infer much specific information from the spectra of the complex mixture. The same is true of ultraviolet spectrometry. High resolution mass spectrometry (HRMS) shows promise for being able to obtain a great deal of specific chemical information even from still very complex mixtures.

Using HRMS, the following classes of compounds have been identified in a solvent extract of the low molecular weight effluent fraction:

fatty acids, aromatic acids, alkylphenylethylene oxides, chlorinated phenols, aliphatic and aromatic amines, and amides.

This report was submitted in fulfillment of Project Number 16020FEN, Contract 14-12-886, under the sponsorship of the Water Quality Office, Environmental Protection Agency.

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